Production of L-DOPA by suspension grown cells of Mucuna pruriens.

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SUMMARY.

In vitro cultured plant cells can be utilized for the production of valuable metabolites. Many biochemical and physiological characteristics of *in vitro* cultured plant cells depend on various environmental parameters, such as the composition of the growth medium in which many parameters are represented. This applies to many primary biosynthetic pathways, as well as to the regulation of the secondary metabolism. Therefore, the regulation of the synthesis of a certain metabolite in the cultured plant cells can in principle be manipulated by variations in the extracellular medium. In the first paragraphs of chapter 1 a survey is given of several properties of plant cells that can be manipulated intentionally, together with properties that will change whether this change was intended or not.
In this thesis the production of L-DOPA with *in vitro* grown cells of *Mucuna pruriens* L. was studied. Therefore, in the last paragraphs of chapter 1 an inmpression is given of the place of L-DOPA in the metabolism of higher plants, and information is given on the accumulation of L-DOPA in higher plants in a quantitative way.
In the following chapters, two approaches are described to achieve the production of L-DOPA with suspension grown cells. The endogenous production of L-DOPA and the effect of environmental parameters thereon are studied (chapters 2 and 3). Furthermore, immobilized cells are used for the biotransformation of L-tyrosine into L-DOPA and of N-formyl-tyrosine into N-formyl-DOPA. As an entrapment matrix, calcium alginate was used (chapters 6 and 7).
From suspension cultures, a phenoloxidase was purified and characterized (chapter 4) and in chapter 5 some methods are described to increase the activity of the phenoloxidase in cell suspension cultures.

An approach as outlined above starts with the initiation of callus and cell suspension cultures of *M.pruriens* and the identification of L-DOPA. The induction of callus and cell suspension cultures, together with the identification of L-DOPA by a number of chromatographic methods and by means of mass spectrometry are reported in chapter 2. A comparison is also made between different cell lines with respect to growth and L-DOPA content.

In chapter 3, the variation of the L-DOPA content of a cell line is followed over a period of two years.
The effect of the concentration of the major nutrients sucrose, nitrate and ammonium, phosphate and of the synthetic auxin 2,4-D are studied. Illumination of the cultures and the addition of L-tyrosine to the growth medium were also found to influence the endogenous production of L-DOPA. In the cell suspension cultures L-DOPA is accumulated intracellularly.

From cell suspension cultures of *M.pruriens* a phenoloxidase could be purified. In chapter 4 the purification procedure is described, and the enzyme is characterized with respect to pH optima, molecular weight, kinetic parameters for some substrates and its behaviour in the presence of some inhibitors.

In chapter 5 the effect of nitrogen limitation and of the addition of pFPA and ethionine on the accumulation of L-DOPA and on the activity of phenoloxidase in cell suspension cultures is described.

Nitrogen limitation, as well as the addition of pFPA or ethionine had a negative effect on the accumulation of L-DOPA. However, the phenoloxidase activity in the cell cultures was stimulated by the manipulations mentioned above. This effect could be utilized in alginate entrapped cells to increase the rate of synthesis of L-DOPA from the direct precursor L-tyrosine, when the cells were treated with pFPA prior to entrapment.

When L-tyrosine is transformed into L-DOPA by alginate entrapped cells of M.pruriens, the L-DOPA is released into the surrounding medium by the plant cells. The composition of the medium in which the biotransformation is performed is not very critical, which offers the possibility to use a very simplified medium (chapter 6).

In chapter 7 the biotransformation of L-tyrosine into L-DOPA, and also the biotransformation of N-formyl-tyrosine into N-formyl-DOPA are further optimized in terms that are relevant for the enzymatic transformation and parameters that are relevant for the entrapped cell system.

In chapter 8 the results as described in this thesis with respect to the endogenous production of L-DOPA are compared to the results obtained by other research groups with the same or a closely related species.
A possible explanation is given for the 'release effect' as described in chapter 6.
Finally, the performance of the endogenous production of L-DOPA by the cell suspension cultures and of the biotransformation by alginate-entrapped cells are compared.